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EUCALYPTUS- A LIGNOCELLULOSIC BIOMASS WASTE AS A MAJOR SOURCE IN BIOETHANOL PRODUCTION

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ABSTRACT

Bioconversion of lignocellulosic biomass like eucalyptus leaves to ethanol production depends on the chemical complexity of biomass. Thus, such biomasses play a challenge to be used as feed stocks for cellulosic ethanol production. The complexity of the biomass can be simplified by pretreatment which may remove or modify the surrounding matrix of lignin and hemicellulose prior to the enzymatic hydrolysis of the polysaccharides in the biomass. Pretreatment is a process that converts lignocellulosic biomass from its native form to a form were cellulose hydrolysis is much more effective. In general, pretreatment methods can be classified into three categories, including physical, chemical, and biological pretreatment. The present investigation deals with the isolation of commercially important bacteria from marine sources which have the potential to breakdown lignocellulosic biomasses. The isolates were screened and the organism exhibiting maximum biodegradation capacity against the substrate was identified as Bacillus pumilus. Different agrowaste were degraded using the isolate and eucalyptus was found to be one of the substrates having the potential to yield high amount of simple sugars which could be used for the production of ethanol by S. cerevisiae. This ability of degradation was enhanced by the pretreatment of Eucalyptus leaves in preparation for enzymatic hydrolysis and microbial fermentation for cellulosic ethanol production.

Key Words: Bioethanol, Lignocellulose, Cellulase, Eucalyptus, Bacillus pumilus.

INTRODUCTION

Earth has the largest Ecosystem in the form of Oceans and 90% of its biomass is microbial. The diversity of microbial life in the oceans is extremely high and spans all known groups of Bacteria, Archaea and microbial eukaryotes. Marine bacteria transform carbon, nitrogen, sulphur and iron compounds, thus playing crucial roles in the global material cycle.

The total world demand for oil is projected to rise by 1% every year mostly due to increasing demand in emerging markets, especially India (3.9%/year) and China (3.5%/year). In view of continuously rising petroleum costs and dependence upon fossil fuel resources, considerable attention has been focused on alternative energy resources. Production of ethanol or ethyl alcohol (CH3CH2OH) from biomass is one way to reduce both the consumption of crude oil and environmental pollution (Lang et al., 2001). Bioethanol represents one of the most prominent technical options due to the possibility of blending it with fossil fuels and using in the existing automobiles without significant adaptations. Unlike fossil fuels, ethanol can be obtained from renewable

source through fermentation of sugars. Ethanol is widely used as a partial gasoline replacement in the USA. Fuel ethanol that is produced from corn has been used in gasohol or oxygenated fuels since the 1980s. These gasoline fuels contain up to 10% ethanol by volume. Ethanol has a higher octane (ability to resist compression) rating than gasoline, enabling combustion engines to run at a higher compression ratio and thus giving a superior net performance (Wyman, 1996).

Lignocellulosic "waste" is generated through forestry and agricultural practices, paper-pulp industries, timber industries and many agro industries and they pose an environmental pollution problem. Most of the lignocellulose waste is often disposed off by burning (Levine, 1996). However, the huge amounts of residual plant biomass considered as "waste" can potentially be converted into various different value added products including biofuels, chemicals, improved animal feeds and human nutrients.

In the 1970s, Brazil and the USA started mass production of bioethanol -from sugarcane and corn respectively. Smaller scale production started more recently from lignocellulosic feedstock derived from agricultural residue. However, lignocellulosic biomass requires a more complicated hydrolysis stage. The reason for this is that complex in the wood contains carbohydrate polymers called cellulose. Cellulose is made up of long chains of glucose and a more complex set of enzymes are required to break the long chains. Therefore, lignocellulosic bioethanol is technically more demanding and thus more expensive.

METHODOLOGY

Isolation and Screening of Microorganisms: Standard microbiological methods were followed for the purpose of isolation of Bacteria from the Cuddalore marine samples (Brown, 1985). Serial dilution of the sample was carried out for isolation of Bacteria on marine agar. The inoculated media plates were incubated at 28oC for 24-48 h.

Screening the organisms for production of cellulase, hemicellulase and ligninases under culture conditions: All of the isolated organisms were subjected to screening for the production of cellulases (Pointing et al. 1999a), hemicellulases (Beily et al., 1985 and Cai et al., 1994) and ligninases (Buswell et al.,1996) on chemically purified substrates CMC, Xylan and Lignin to check for degradation. The isolate which exhibited maximum lignocellolysis was identified using basic microbiological techniques, biochemical test and molecular analysis as Bacillus pumilus.

Substrate Optimization:
Eighteen various kinds of Agro waste, domestic and industrial waste from rural parts of Bangalore were subjected to biodegradation by Bacillus pumilus. Eucalyptus was found to be one of the agro waste which yielded maximum amount of reducing sugars. To enhance the yield of reducing sugars which could ultimately be used for bioethanol production the substrate was prê treated with different physical and chemical parameters.

Optimization of Physical parameters :

pH: The eucalyptus leaves were cut into small pieces and transferred to flasks containing water. Different pH 4, 5, 6, 7, 8 and 9 were set using 1N NaOH and 1N HCl (Pezsa and Ailer, 2011). The flasks were sterilised and inoculated with isolated culture of Bacillus pumilus. The amount of reducing sugar was estimated using DNS (Miller, 1959) method and lignin degradation was carried out using veratryl alcohol assay. The DNS and veratryl alcohol assay were measured at an interval of seven days for 8 consecutive weeks.

High temperatures: The eucalyptus leaves were cut into small pieces and added in flasks containing 100ml of water and kept at different temperatures i.e., 1000C, 1500C, 2000C and 2500C for 1hr (Brownell and Saddler, 1986) by placing them in hot air oven which were set for the temperature as given before. Once the pre treatment was carried out and flasks had cooled, they were inoculated with the strain of *Bacillus*

pumilus. The reducing sugar was estimated using DNS (Miller, 1959) and lignin degradation using veratryl alcohol (Frederick, 1992).

Incubation Temperature: Eucalyptus leaves were cut into small pieces and added into flasks each containing 100ml of water sterilised and they were inoculated with Bacillus pumilus. After inoculation, the flasks were incubated at 4 different temperatures to study the impact of the incubation temperature on degradation of lignocellulosic biomass. The temperatures at which the incubation was done were 25, 30, 37 and 40°C (Charitha Devi et al., 2012). Reducing sugar was estimated using DNS (Miller 1959) and lignin degradation using veratryl alcohol (Frederick, 1992) to check the degradation of lignocellulosic biomass. The readings were taken at regular interval of 7 days for 8 consecutive weeks to check the impact of the incubation temperature.

Optimization of Chemical parameters :

Carbon source: 5 different carbon sources were selected for the study glucose, maltose, starch, dextrose and fructose. Different concentrations i.e., 0.5%, 1.0%, 1.5% and 2.0% (Mehdi Dashtban et al., 2011) of each source were taken into flasks. Eucalyptus leaves were cut into small pieces and added into flasks containing water with different concentrations of carbon sources - 0.5%, 1.0%, 1.5% and 2.0% . The flasks were then autoclaved and then inoculated with Bacillus pumilus. The reducing sugar was estimated using DNS (Miller, 1959) and lignin degradation using veratryl alcohol (Frederick, 1992). The readings were further taken weekly for 8 consecutive weeks.

Nitrogen source: 5 different Nitrogen sources were selected for the study ammonium nitrate, ammonium sulfate, urea, sodium nitrate and potassium nitrate. Different concentrations (0.5%, 1.0%, 1.5% and 2.0%) of each source were taken into flasks. Eucalyptus leaves were cut into small pieces and was added into flasks containing water with different concentrations of nitrogen sources - 0.5%, 1.0%, 1.5% and 2.0% and inoculated with Bacillus pumilus. The reducing sugar was estimated using DNS (Miller 1959) and lignin degradation using veratryl alcohol (Frederick, 1992). The readings were further taken weekly for 8 consecutive weeks.

Acid Treatment: Eucalyptus leaves were cut into small pieces and added into flasks containing different concentrations of acid 0.1%, 0.3%, 0.5%, 0.7%, 0.9% and 1% (Leenakul and Tippayawong, 2010, Nutawan et al., 2010). The flasks were incubated at room temperature for 24 hours. The substrates were then neutralized (Umbrin et al, 2011) and sterilised then were inoculated with Bacillus pumilus. Reading was taken for reducing sugar using DNS (Miller, 1959) and lignin degradation using veratryl alcohol. The readings were taken weekly for 8 consecutive weeks.

Alkali Treatment: Eucalyptus leaves were cut into small pieces and added into flasks containing different concentrations of alkali - 0.1%, 0.3%, 0.5%, 0.7%, 0.9% and 1% (Ashish vyas et al,. 2005). The flasks were left at room temperature for 24 h. The substrates were then neutralized and inoculated with Bacillus pumilus. Reading was taken for reducing sugar using DNS and lignin degradation using veratryl alcohol assay.

Statistical Analysis:

The statistical analysis of the optimization and process parameters results was done using Microsoft Excel and Standard deviation. The readings for all the individual optimization conditions were documented and used further for optimization in the production of bioethanol.

Production of Bioethanol:

Eucalyptus leaves were pre treated under the optimal conditions overnight with 0.5% alkali, high temperature treatment of 200^0C for 1 h. The substrate was then suspended in distilled water and the flasks were sterilized and optimized amount of carbon (maltose- 2%) and nitrogen (ammonium nitrate- 2.0%) sources were added and the pH was set at 4.0. The flasks were inoculated with Bacillus pumilus (Accession number: JQ428828) and incubated at 30^0C for

30 days. The incubated substrates were taken and filtered with 0.45 micron Whatmann filter paper by using filtration unit (Fatma, 2010). The filtrate was collected separately and inoculated with 3% Saccharomyces cerevisiae. The Saccharomyces cerevisiae inoculated flasks were incubated at 30°C for 40 days. Samples were drawn on 10th, 20th, 30th and 40th day and distillation was carried out. The amount of alcohol was estimated by potassium dichromate method.

Gas Chromatography Analysis:
The confirmation of ethanol qualitatively and quantitatively was done by Gas Chromatography Analysis using standard ethanol.

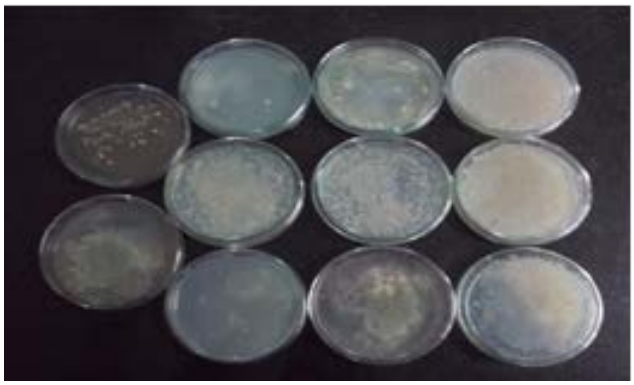
RESULTS AND DISCUSSION

A total of 39 bacterial isolates were obtained from Cuddalore marine samples (Plate 1) and all the isolated organisms were subjected to screening for the production of cellulases, hemicellulases and ligninases on pure substrates such as Carboxy Methyl Cellulose (CMC), Xylan and Lignin. Bacillus pumilus (Accession number: JQ428828) showed highest zone of degradation for all the three substrates. Eucalyptus was found to give the highest quantity of sugars when compared to other 17 substrate used in the experiment. Thus, the optimisation of the various physical and chemical parameters was carried out for Eucalyptus and Bacillus pumilus.

A diverse spectrum of lignocellulolytic bacteria (McCarthy, 1987; Zimmermann, 1990; Vicuňa, 1988) have been isolated and identified over the years and the list continues to grow. The screening activity which was assessed based on dye staining and zone of hydrolysis similar to the findings of wood et al. (1988) and Rubeena et al. (2013). The Gram positive Bacillus that exhibited lignocelluloytic ability was on par with the bacteria reported by König et al. (2005)

Bacillus pumilus exhibited maximal degradation of cellulose in Eucalyptus at pH 4 in the 5th week (Figure 1), incubation temperature at 30°C in the 5th week (Figure 2), acid hydrolysis 0.9% in the initial reading(Figure 4), alkali hydrolysis 0.5%in the 1st week (Figure 5), high temperature treatment at 200°C in the 6th week(Figure 3), among the nitrogen source(Figure 6-10), ammonium nitrate was found to be best nitrogen source at 2% in the 6th week(Figure 6) and the variation of carbon source such as maltose (Figure 11), sucrose (Figure 12), lactose (Figure 13), starch (Figure 14) and fructose (Figure 15) at 2% concentration in the 3rd week having no increased impact in degradation.

Plate 1: Bacterial isolates



Bacillus pumilus exhibited maximal degradation of lignin in Eucalyptus at pH 6 in the 1st week (Figure 1) , incubation temperature at 40°C in the 7th week (Figure 2), acid hydrolysis 0.1% in the initial reading (Figure 4), alkali hydrolysis 1.0 %in the initial reading (Figure 5), high temperature treatment at 150°C in the 4th week (Figure 3), among the nitrogen source(Figure 6-10) ammonium nitrate was found to be the best nitrogen source at 1.0% in the 2nd week (Figure 6) and the variation of carbon source such as maltose (Figure 11), sucrose (Figure 12), lactose (Figure 13), starch (Figure 14) and fructose (Figure 15) at 1.5% concentration in the 1st week having no increased impact in degradation.

Pre-treatment of various substrate with 1% (1N NaOH) Alkali proved to be sufficient to obtain maximum delignification, enhancing lignocellulase activity which varies with the results obtained by Acharya et al. (2008) where they pretreated saw dust with 2N NaOH and obtained maximum cellulase activity at 9.6% concentration, but is on par with the results of

Figure 1: Optimization of pH indicates that maximum reducing sugar and lignin oxidation is at pH 4 in 5th week and pH 6 in the 1st week.

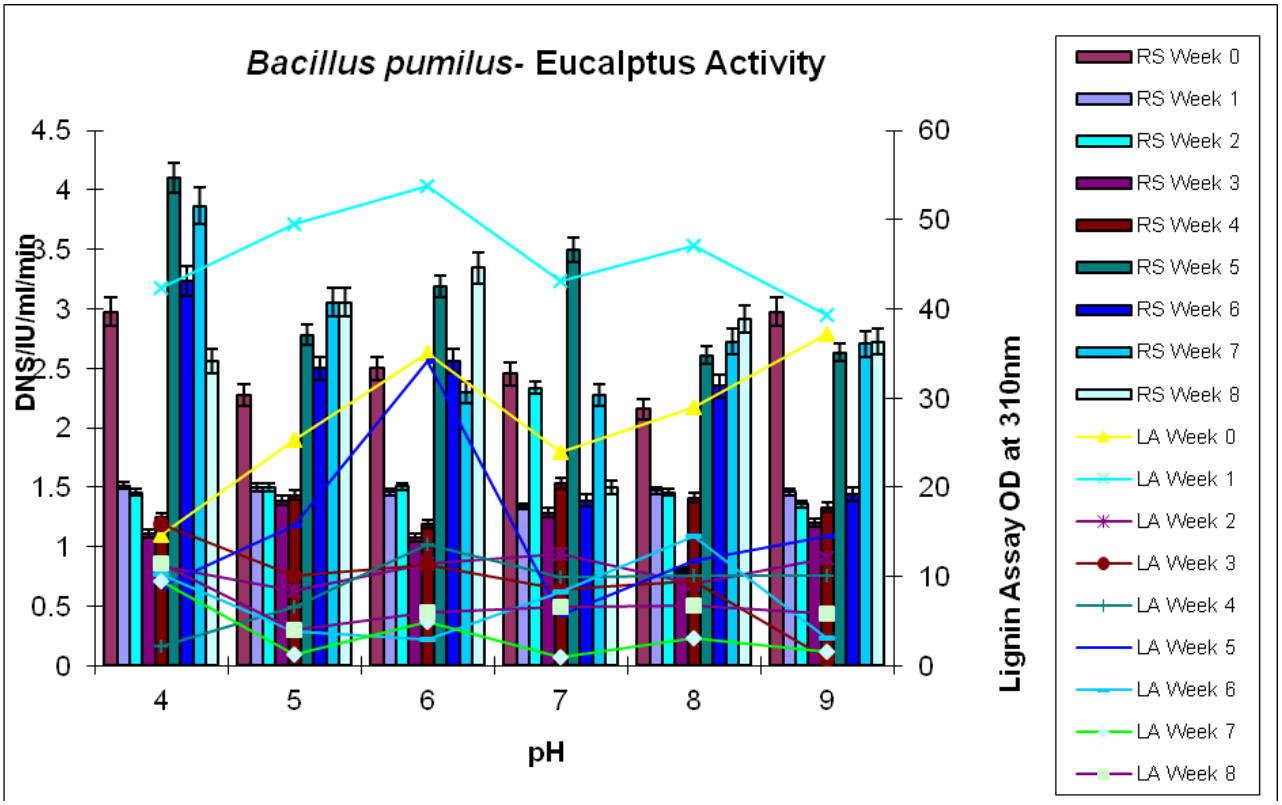


Figure 2: Optimization of Incubation Temperature indicates that maximum reducing sugar and lignin oxidation is at 30^{0}C in 5th week and 40^{0}C in the 7th week.

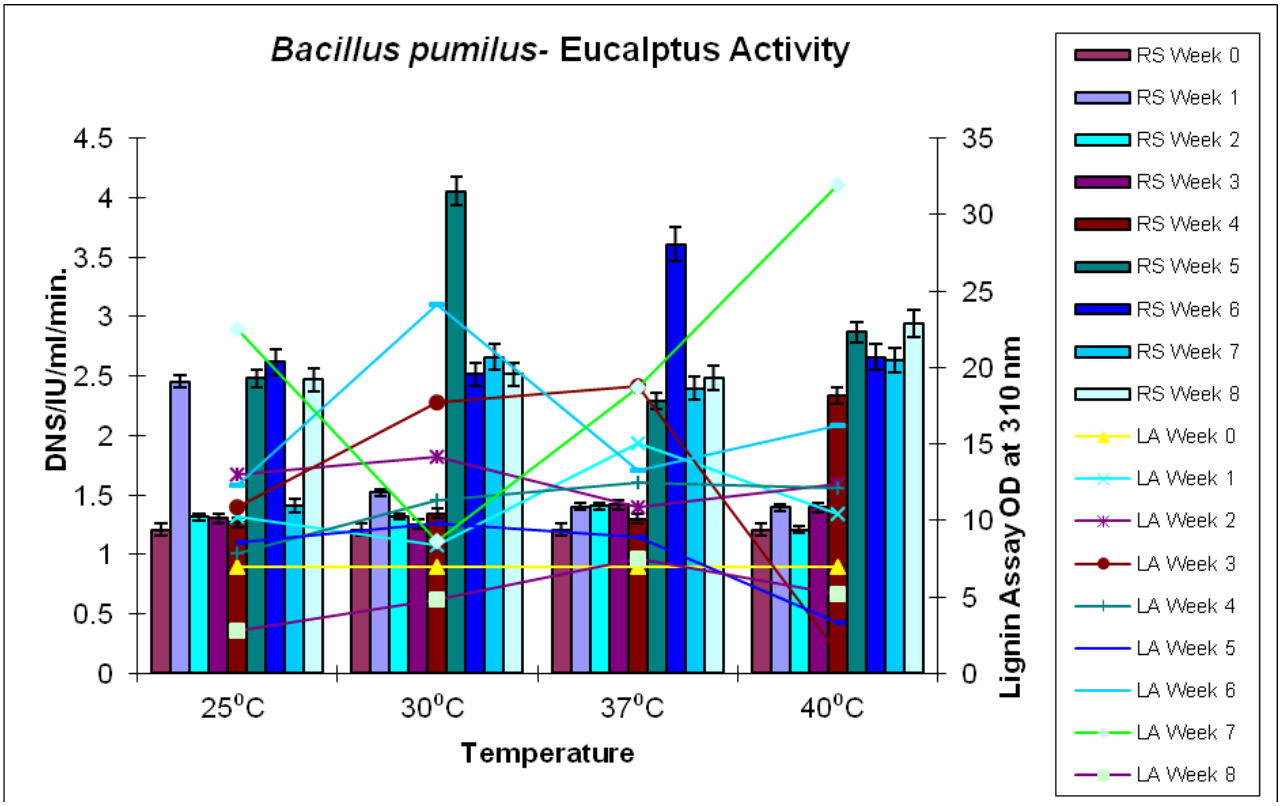


Figure 3: Pre Treatment with High Temperature indicated highest yield of reducing sugar at 200^{0}C in 6th week and lignin oxidation at 150^{0}C in 4th week

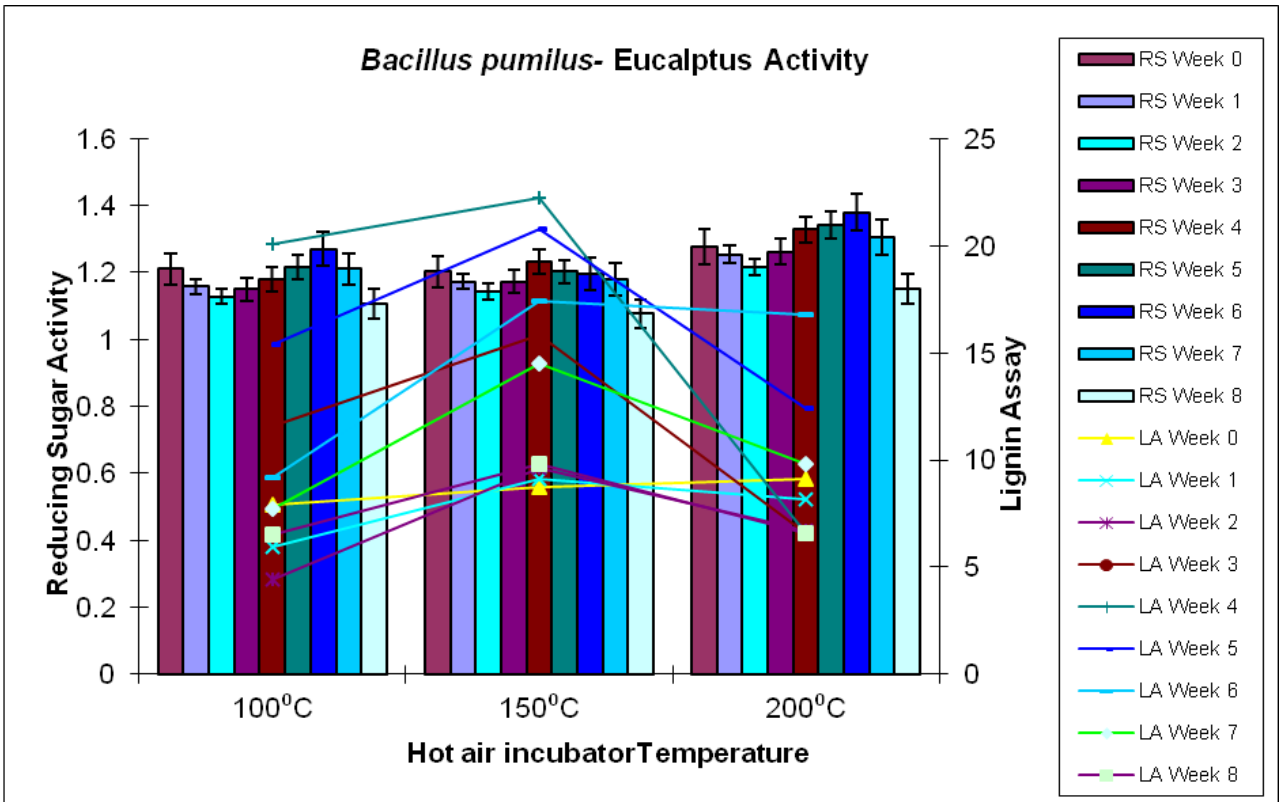


Figure 4: Pre Treatment with Acid indicates highest reducing sugar at 0.9 % and lignin oxidation at 0.1 % in the initial reading.

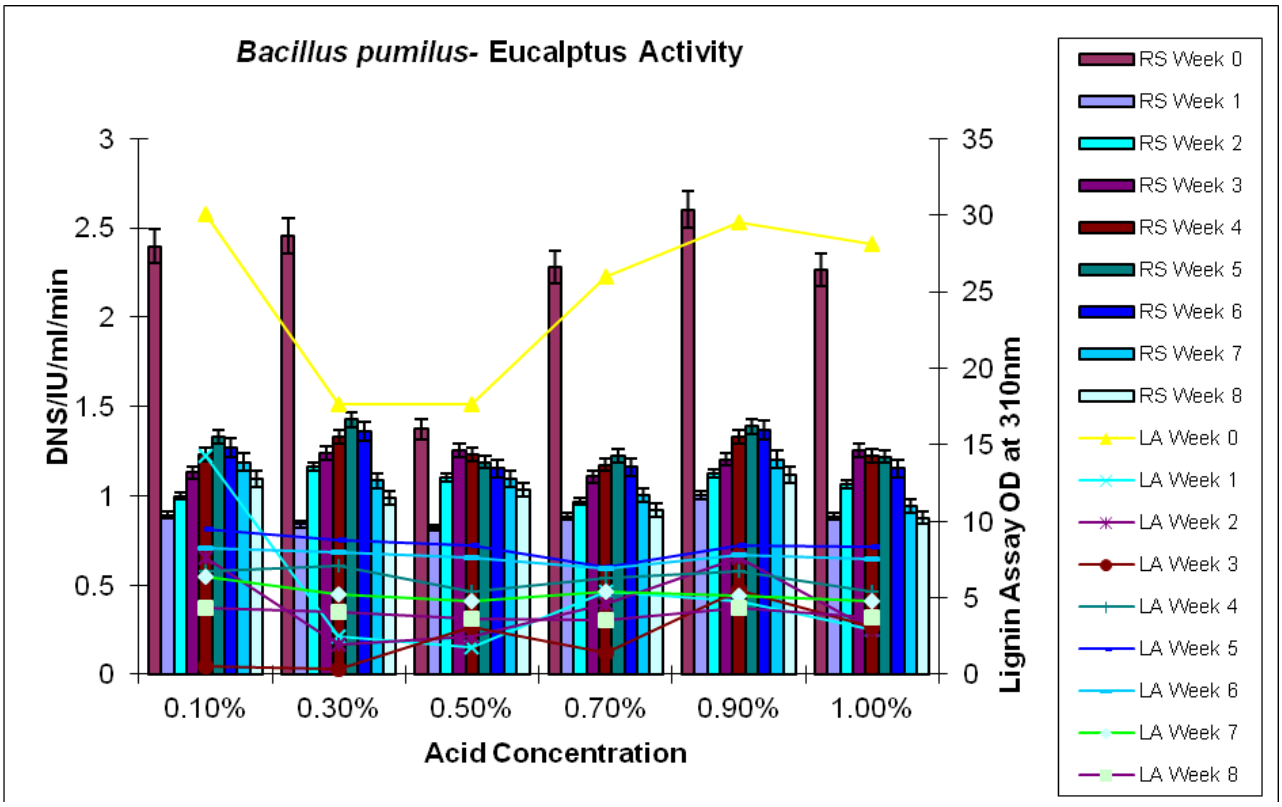


Figure 5: Pre Treatment with Alkali indicates highest reducing sugar at 0.5 % in 1st week and lignin oxidation at 1.0 % in the initial reading.

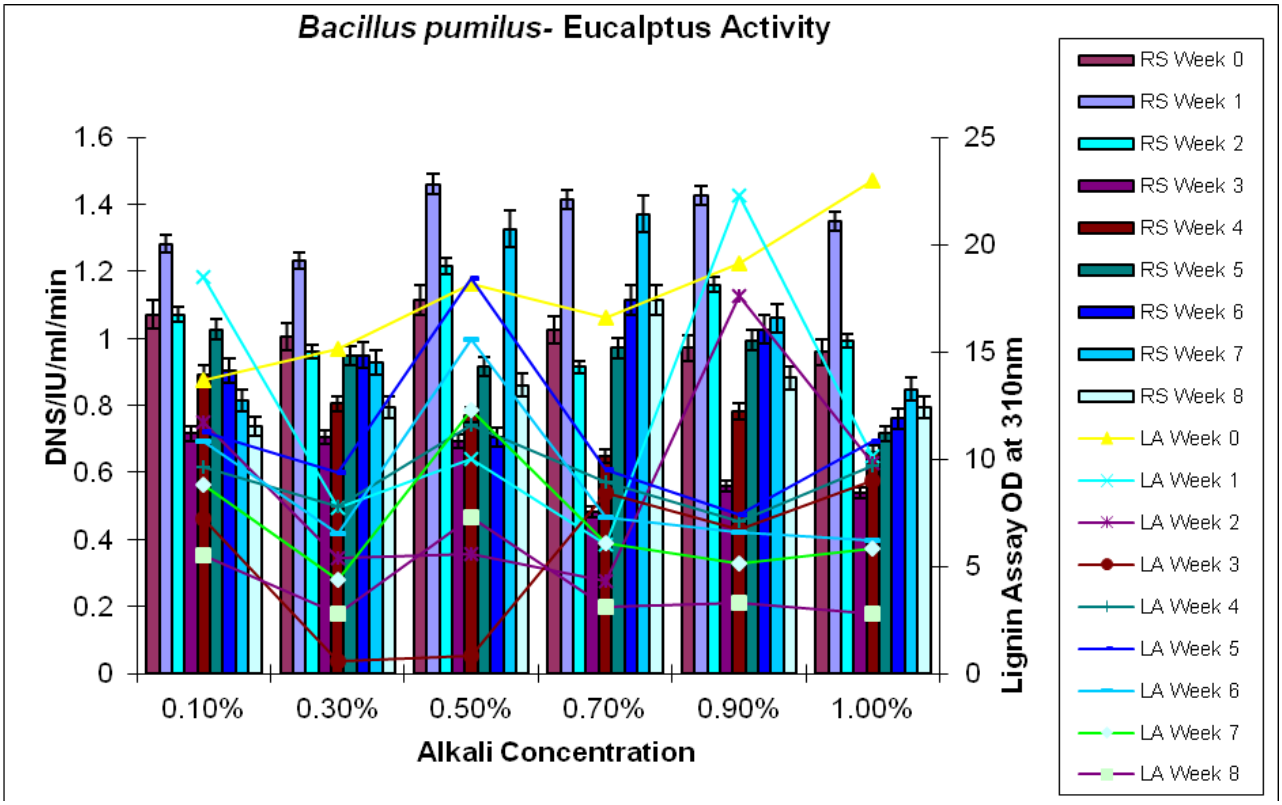


Figure 6: Optimization with Nitrogen source: Ammonium Nitrate indicates highest yield of reducing sugar at 2.0 % in 6th week and lignin oxidation at 1.0% in the 2nd week.

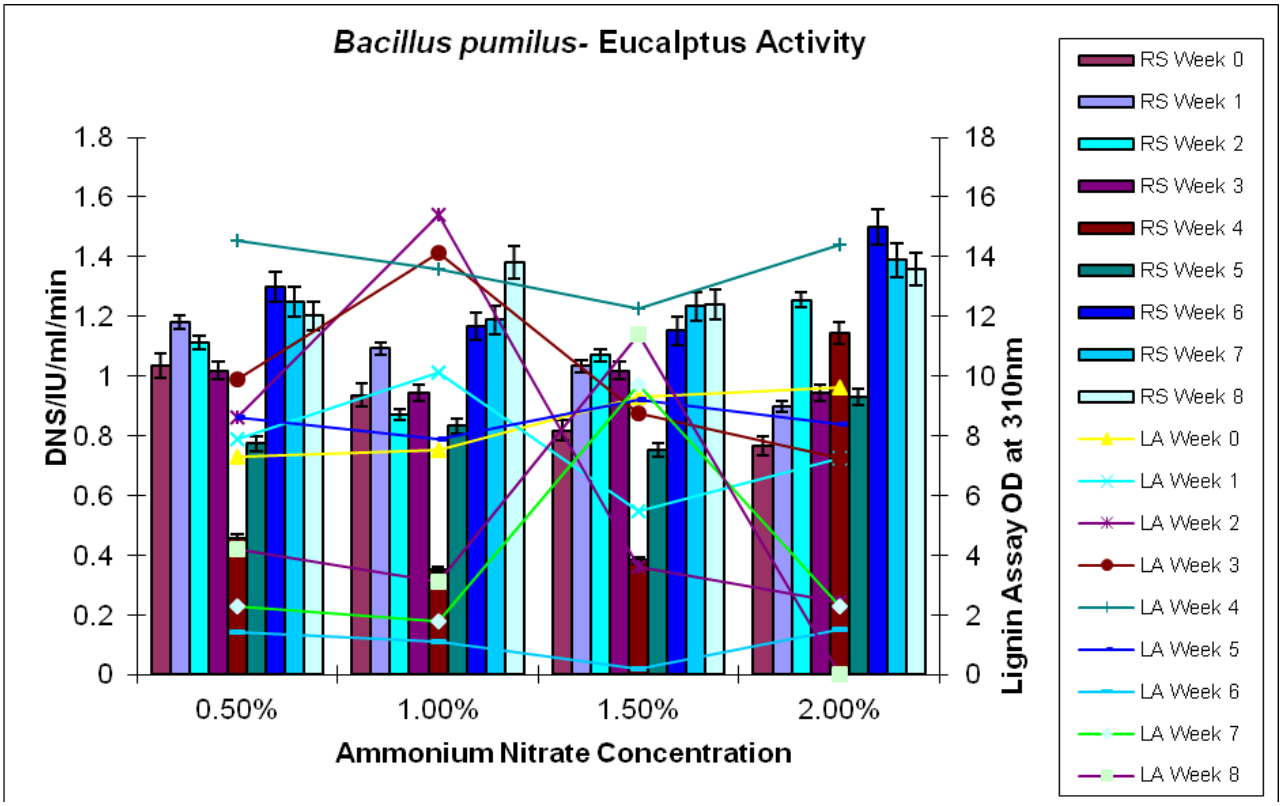


Figure 7: Optimization with Nitrogen source: Ammonium Sulphate indicates highest yield of reducing sugar at 0.5 % and lignin oxidation at 2.0% in the 5th week.

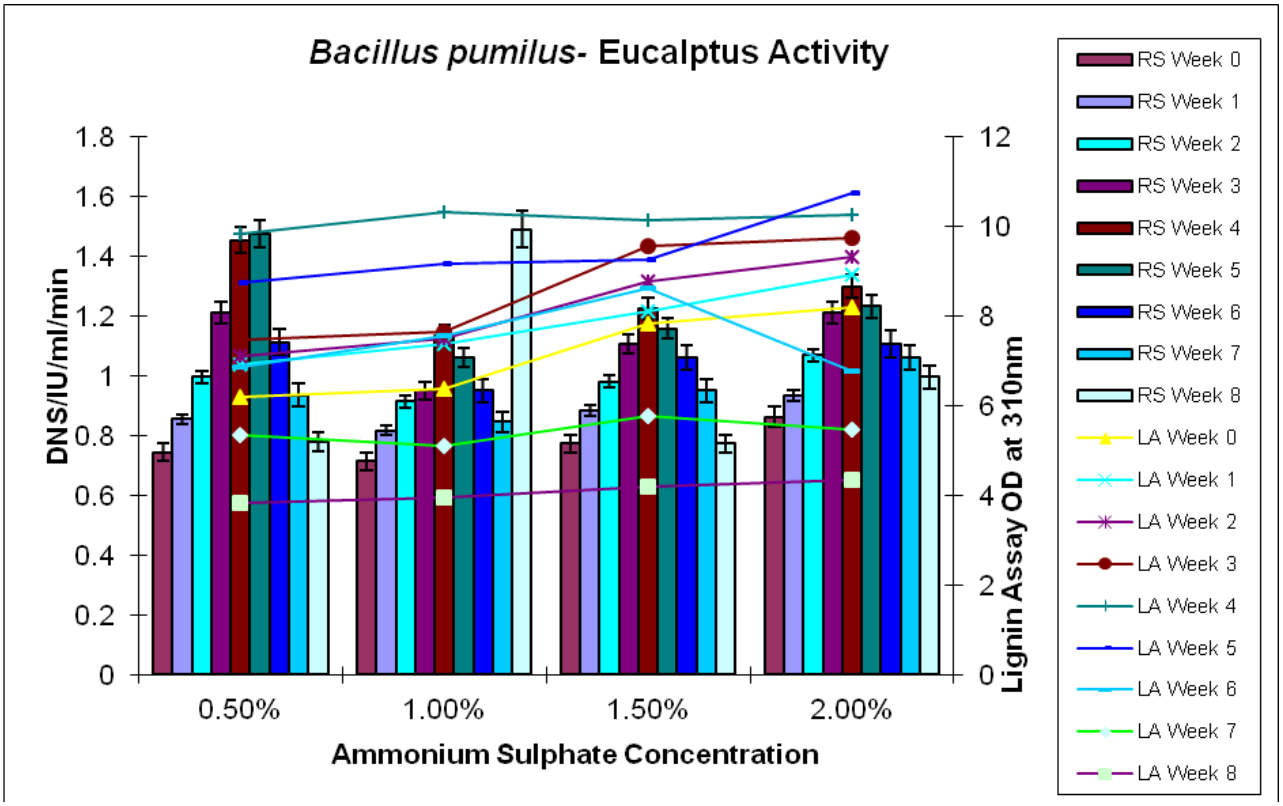


Figure 8: Optimization with Nitrogen source: Urea indicates highest yield of reducing sugar at 1.5 % and lignin oxidation at 2.0% in the 4th week.

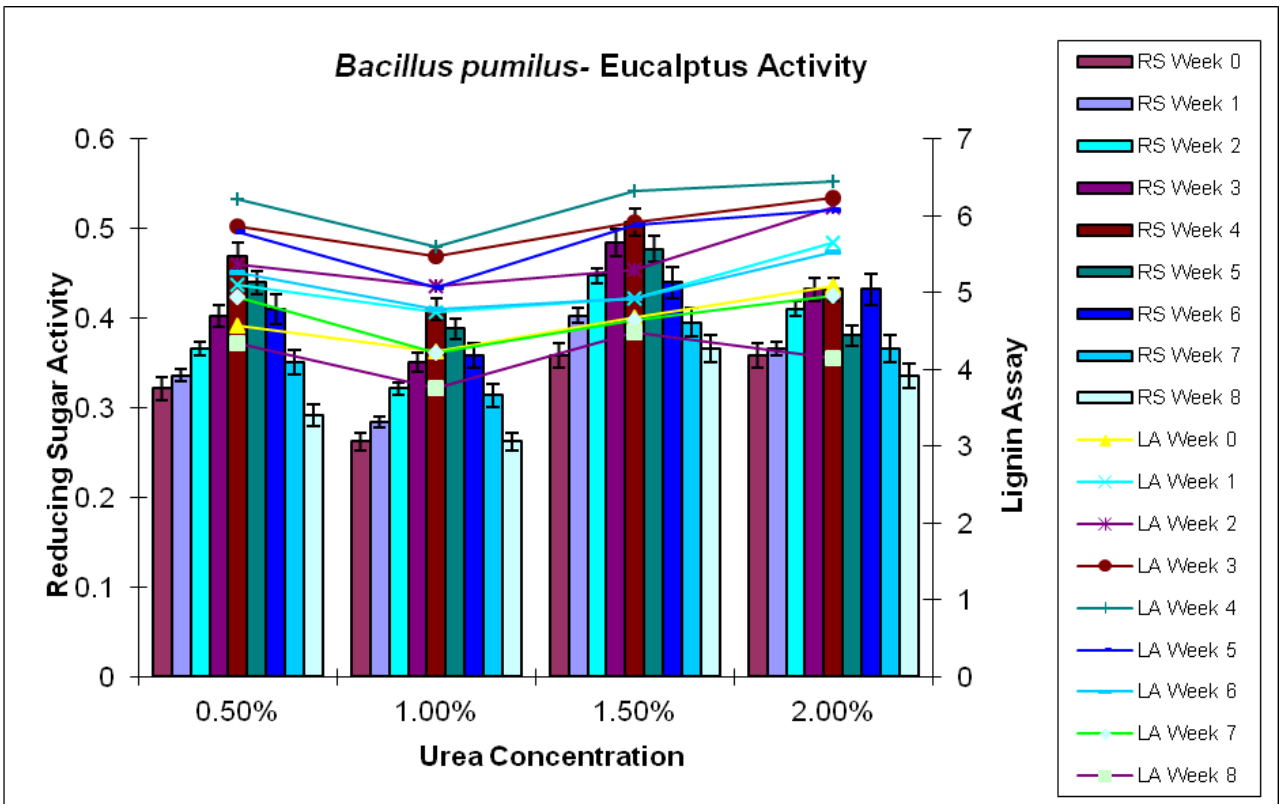


Figure 9: Optimization with Nitrogen source: Ammonium Phosphate indicates highest yield of reducing sugar and lignin oxidation at 2.0% in the 4th week.

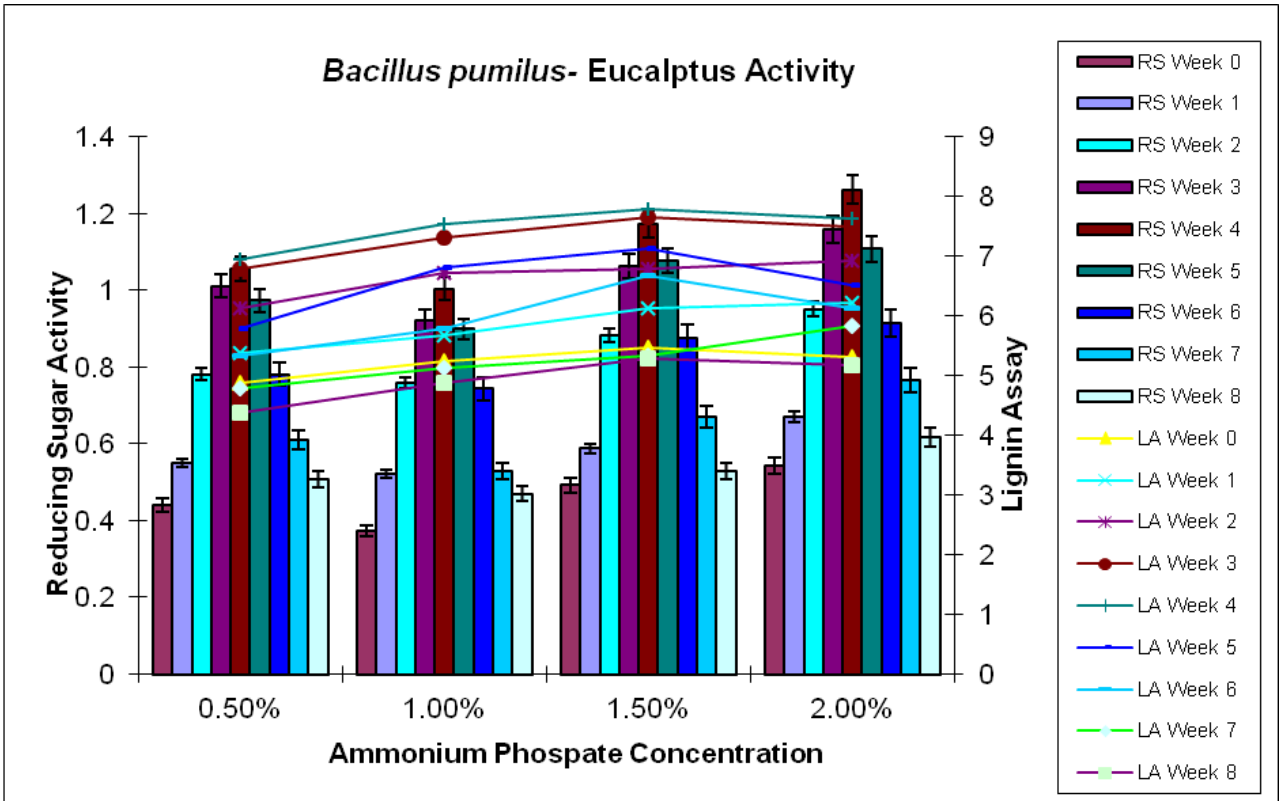


Figure 10: Optimization with Nitrogen source: Sodium Nitrate indicates highest yield of reducing sugar at 2.0 % in 4th week and lignin oxidation at 1.5% in the 3rd week.

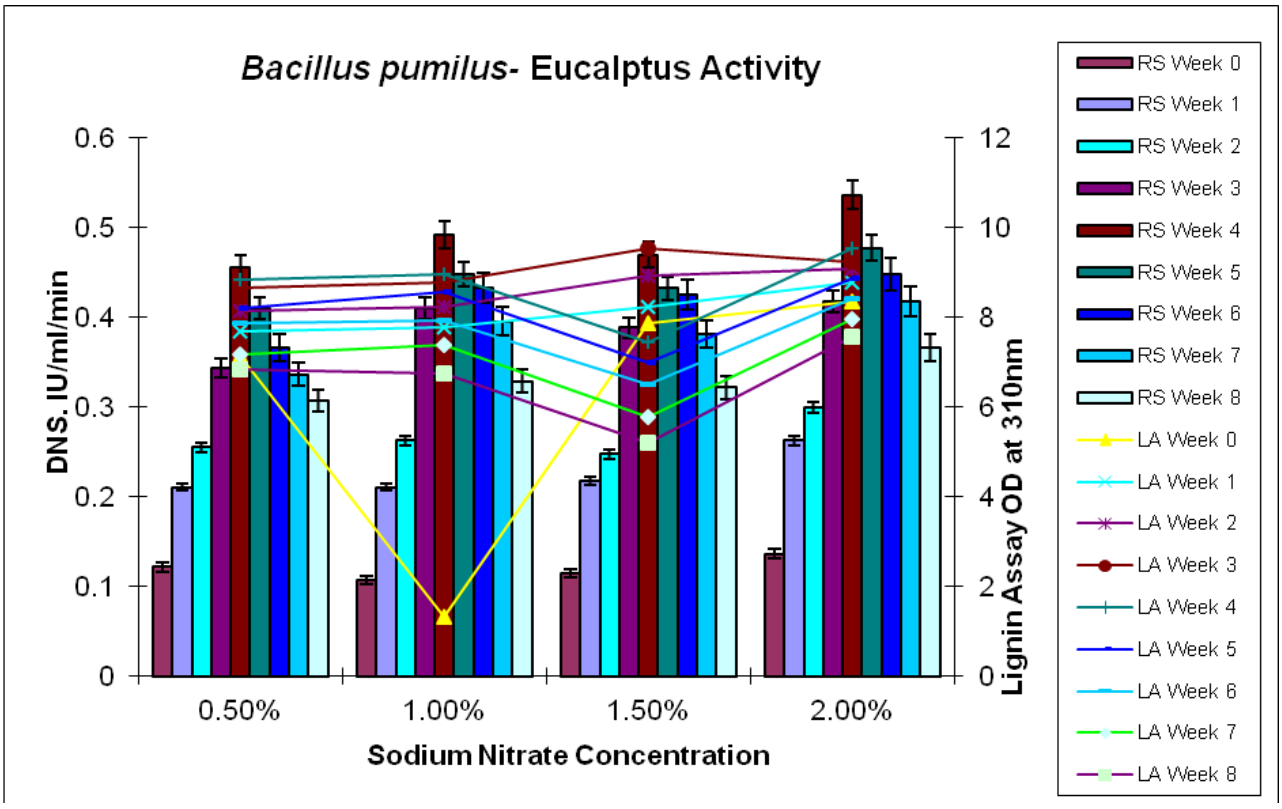


Figure 11: Optimization with Carbon source: Maltose indicates highest yield of reducing sugar at 2.0 % in 3rd week and lignin oxidation at 1.5% in the 1st week.

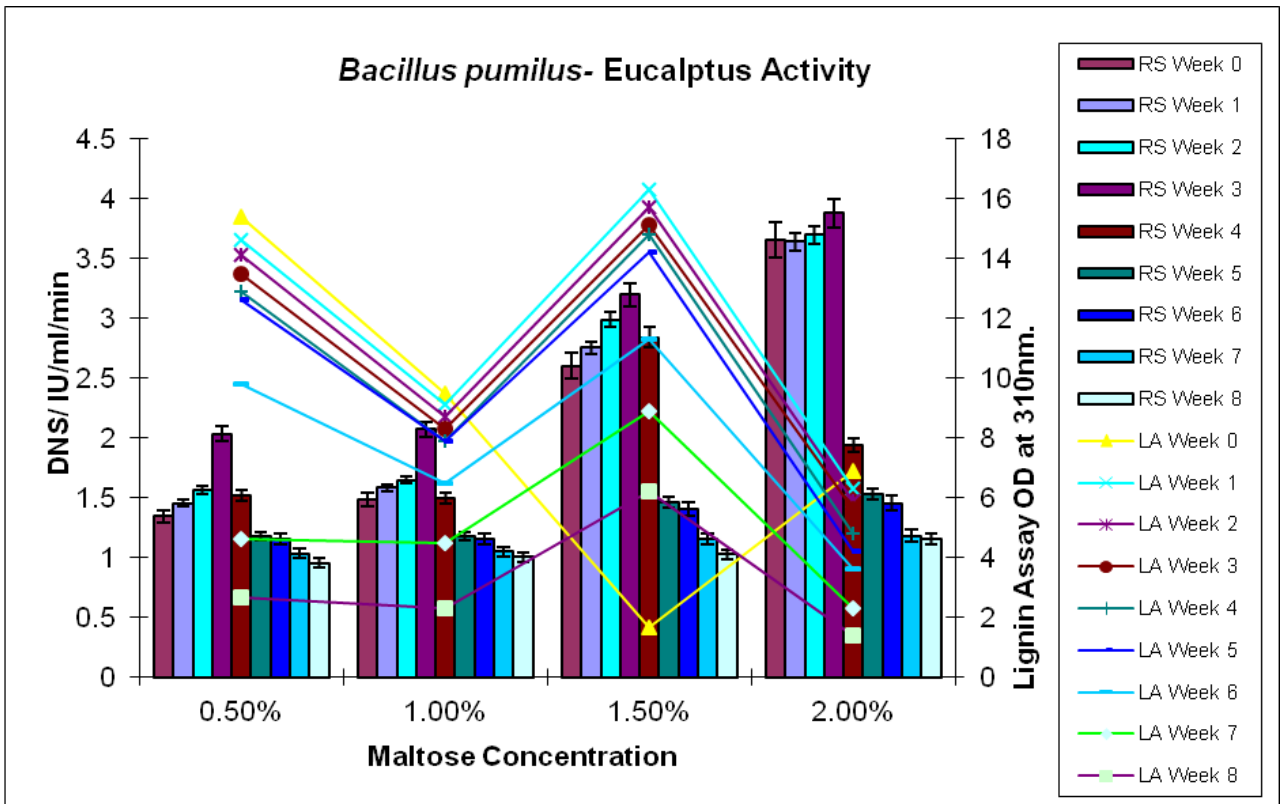


Figure 12: Optimization with Carbon source: Sucrose indicates highest yield of reducing sugar at 2.0% and lignin oxidation at 0.5% in the 4th week.

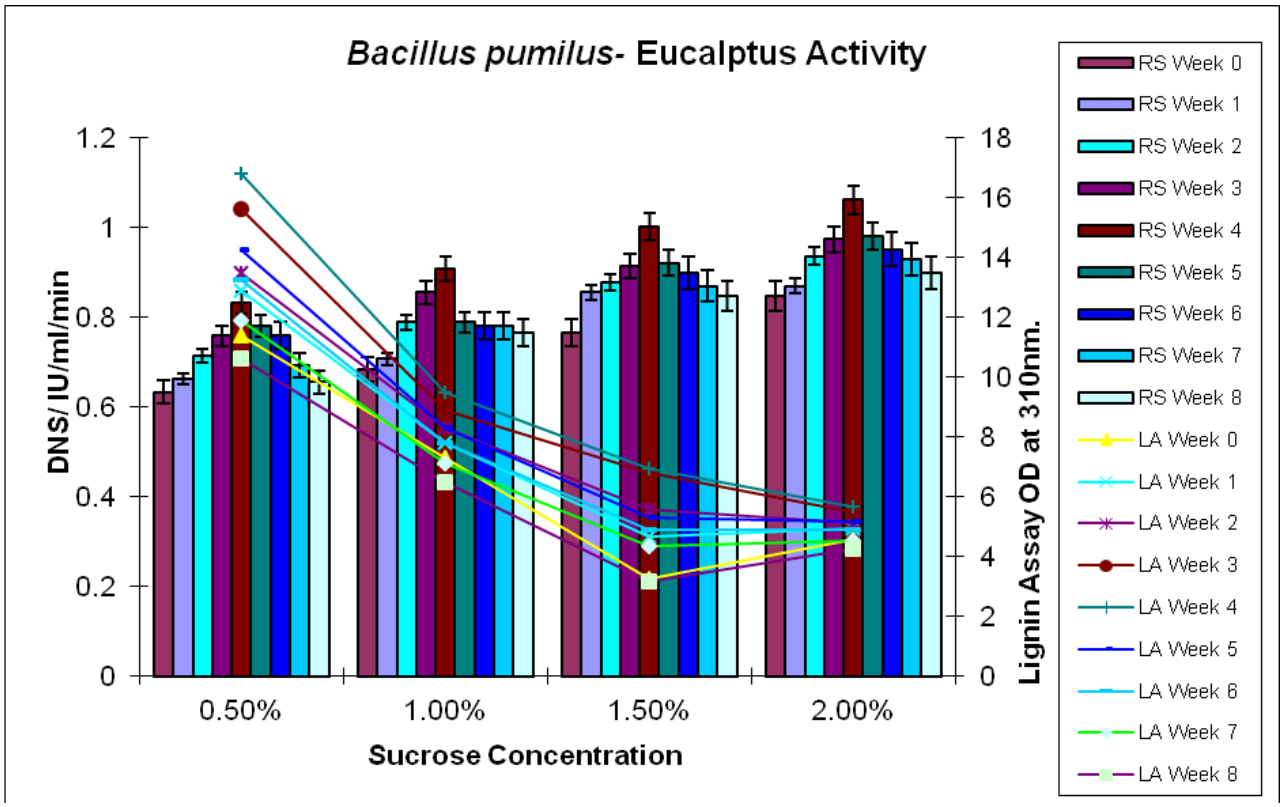


Figure 13: Optimization with Carbon source: Lactose indicates highest yield of reducing sugar at 2.0 % and lignin oxidation at 1.0% in the 4th week.

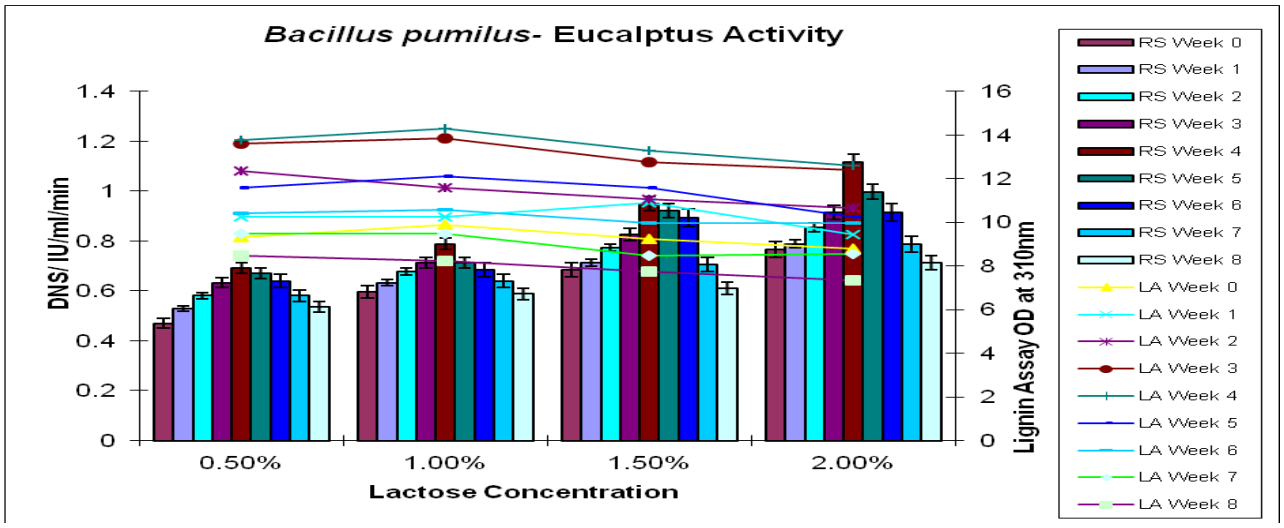


Figure 14: Optimization with Carbon source: Starch indicates highest yield of reducing sugar and lignin oxidation at 2.0% in the 4th week.

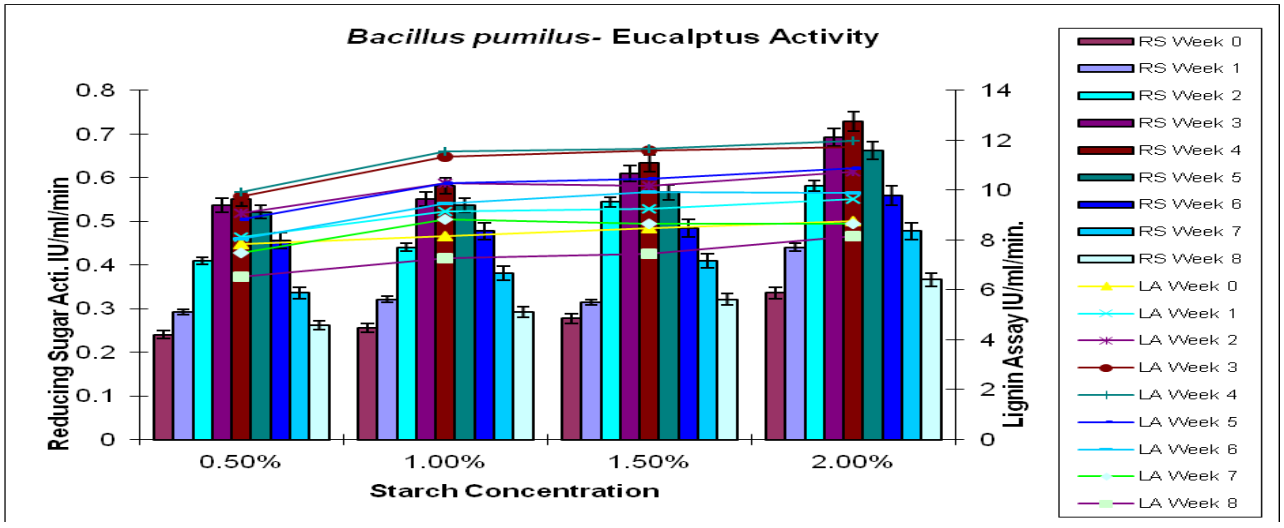


Figure 15: Optimization with Carbon source: Fructose indicates highest yield of reducing sugar at 2.0 % and lignin oxidation at 1.0% in the 4th week.

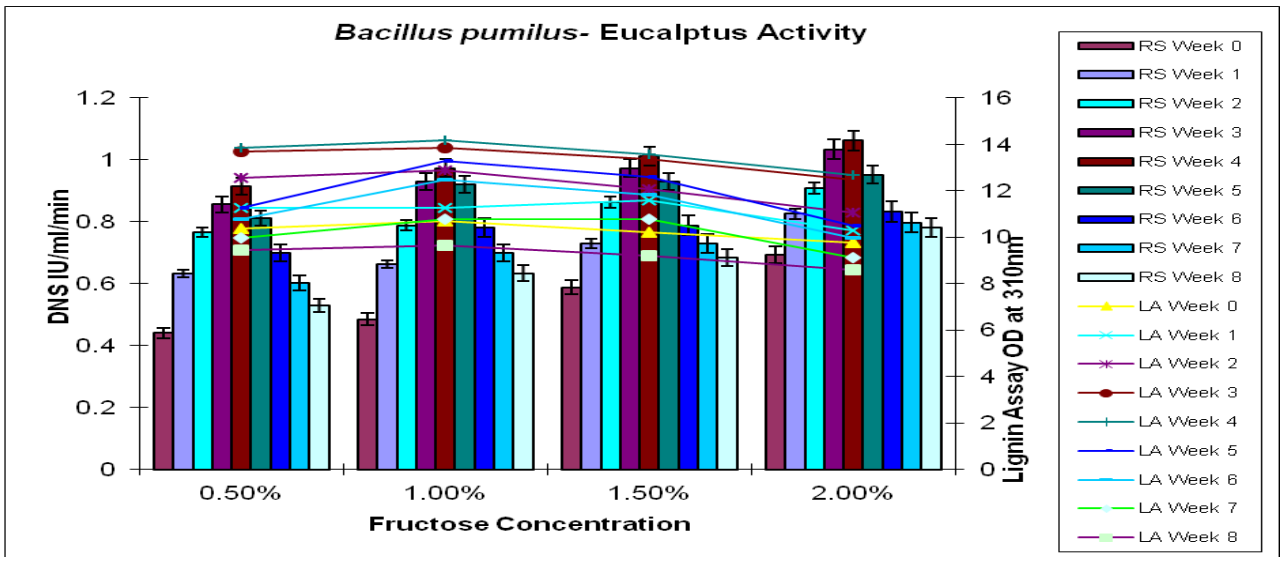


Figure 16: Alcohol Percentage (Bacillus, Eucalyptus)

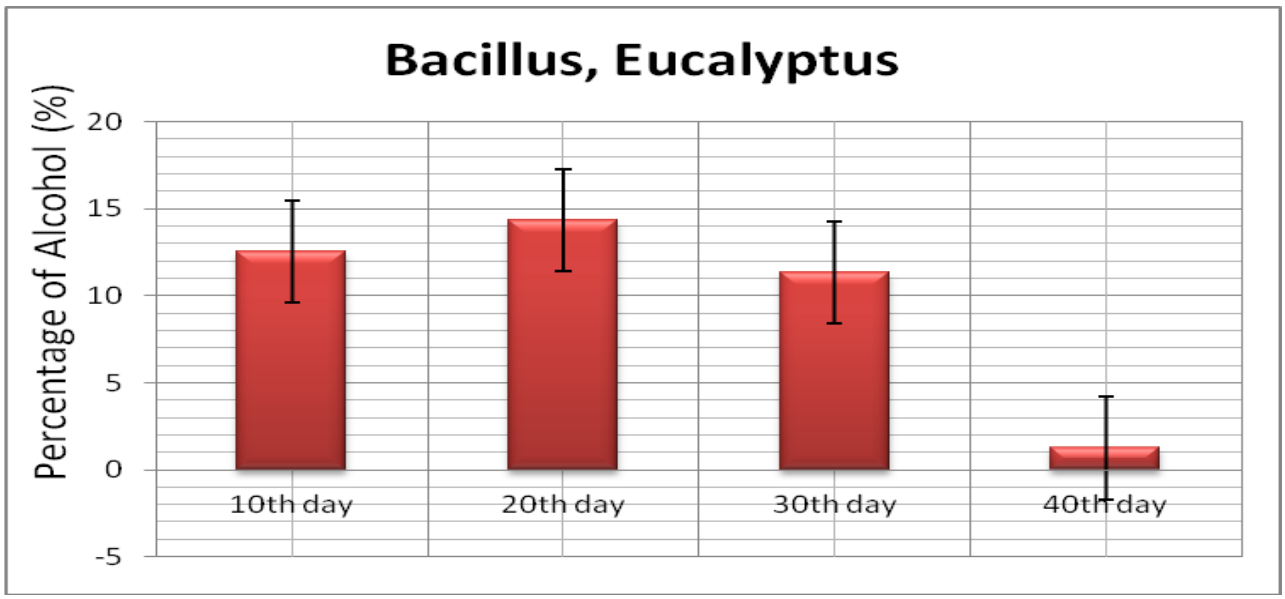


Figure 17: GC of Standard Ethanol

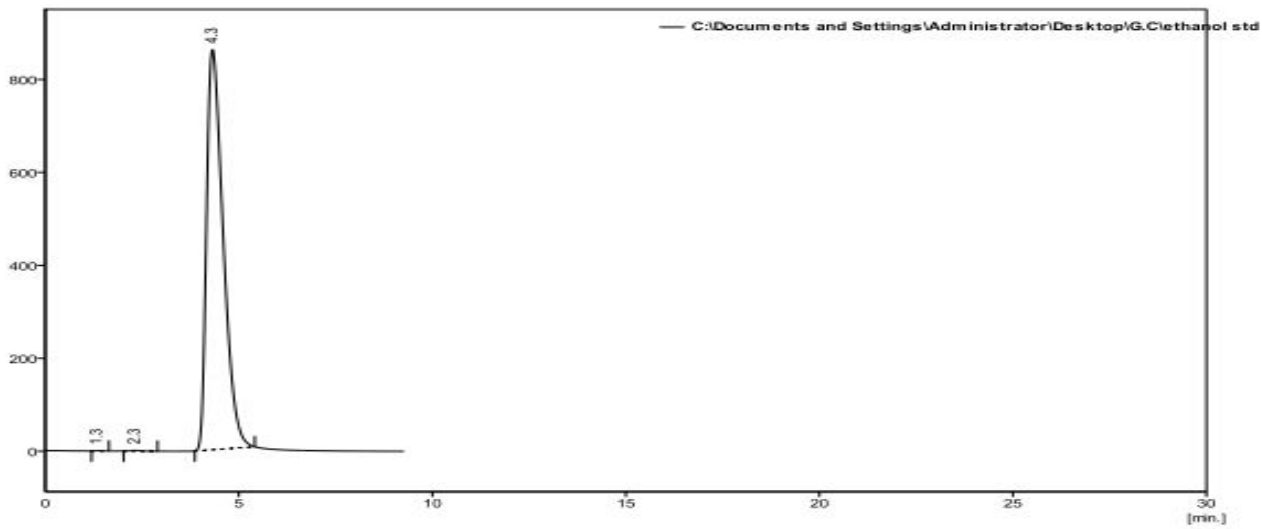
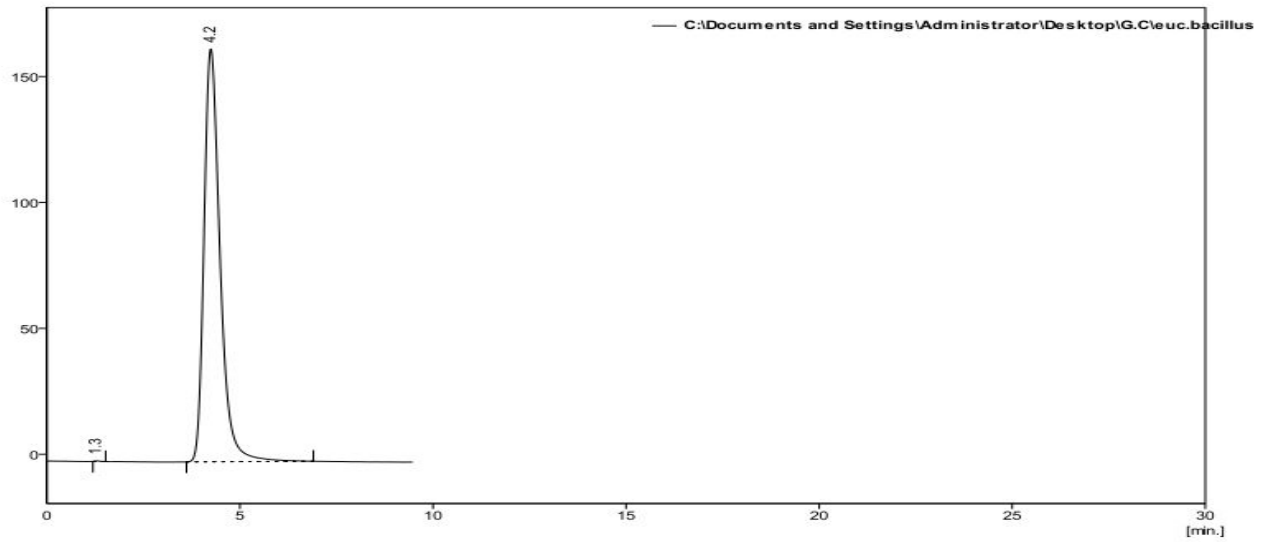


Figure 18: GC of *Bacillus pumilus* with Eucalyptus



Keshwani (2009) carried out on switch grass with the usage of 1% and 3% NaOH.

Variation in the percentage of various nitrogen sources on different substrates proved that 1.5% of ammonium nitrate was supportive to enzymatic hydrolysis which deviated from the results obtained by Narasimha et al. (2006), where urea was the best nitrogen source. The addition of various carbon sources increased the sugar yield and enzymatic hydrolysis in our study which is opposite to the result obtained by Moussa et al. (2007) whose findings reported the repression of cellulases on addition of carbon source. The strategy of combined pretreatment of heat and either alkali/acid has been used in the present study which is similar to the findings of Gupta (2008), Maarten et al. (2009) and Hu et al. (2008).

The filtrate obtained after hydrolysis of eucalyptus leaves was subjected to alcoholic fermentation using Saccharomyces cerevisiae. The alcohol yield was found to be maximum on 20th day with alcohol percentage of 14.35%, the least alcohol percentage was found on 40th day with a percentage of 1.25 (Figure 16). The filtrate obtained on the 20th day was analysed using GC for qualitative and quantitative confirmation of the alcohol. The GC results showed that the alcohol percentage was 17.34% (Figure 18).

The maximum Bioethanol yield was found on the 20th day which varies with the findings of Naresh Sharma et al. (2007) where the maximum yield was found on 2nd day with a combination of two waste. Bacillus pumilus on rice straw yielded maximum sugar by pretreatment with acid which is comparable to the finding of El-Refai et al. (1992). Bioethanol was produced from plentiful, domestic, cellulosic biomass resources such as agro-industrial and domestic residues which is similar to the findings of Ayhan demirbas(2005).

REFERENCES

1. Moussa, Tarek A. and Nagwa A. Tharwat, Optimization of cellulase and _-glucosidase induction by sugarbeet pathogen Sclerotium rolfsii, African Journal of Biotechnology Vol. 6 (8), pp. 1048-1054, 16 April 2007.
2. Acharya P. B., D. K. Acharya and H. A. Modi, Optimization for cellulase production by Aspergillus niger using saw dust as substrate, African Journal of Biotechnology Vol. 7 (22), pp. 4147-4152, 19 November, 2008.
3. Ashish Vyas, Deepak Vyas and K M Vyas. “Production and optimization of cellulases on pretreated ground nut shell by Aspergillus terreus AV 49”.Journal of Scientific and Industrial Research, Vol. 64, April 2005,pp.281-286.
4. AYHAN DEMIRBAS, Bioethanol from Cellulosic Materials:A Renewable Motor Fuel from Biomass, Energy Sources, 27:327-337., 2005.
5. Biely, P. Xylanolytic enzymes. In WHITAKER, J.R.-VORAGEN, A.G.J.-WONG, D.W.S. Handbook of Food Enzymology. New York: Marcel Dekker, Inc., 2003,p. 879-915.
6. Brown,C.M., Isolation methods for Microorganisms, P.(21-35) In, comprehensive Biotechnology ed. In chief-Murray Scientific fundamentals. Howard Dalton.Publ. Pergam press, 1985; Oxford.
7. Brownell, H.H. And Saddler, N.J., , Steam pretreatment of lignocellulosic material for enhanced enzymatic hydrolysis. Biotechnology and Bioengineering, 1986, 29: 228-235.
8. Buswell, J.A., Cai, Y.J., Chang, S.T., Peberdy, J.F., Fu, S.Y. and Yu, H.S. Lignocellulolytic enzyme profiles of edible mushroom fungi. World Journal of Microbiology and Biotechnology. 1996; 12, 537-542.
9. Cai, Y.J., Buswell, J.A. and Chang, S.T. Cellulases and himicellulases of Volvariellavolvacea and the effect of tween 80 on enzyme production. Mycological Research. 1994; 98, 1019-1024.
10. Charitha Devi M. and M. Sunil Kumar.” Production, Optimization and Partial purification of Cellulase by Aspergillus niger fermented with paper and timber sawmill

industrial wastes". J. Microbiol. Biotech. Res., 2012, 2 (1):120-128.
11. El-Refai AH, El-Abyad MS, El-Diwany AI, Sallam LA and Allam RF (1992) Some physiological parameters for ethanol production from beet molasses by Saccharomyces cerevisiae Y-7. Bioresour Technol 42:183–189.
12. Fatma, H. Abd El-Zaher1 and Fadel, M. "Production of Bioethanol via Enzymatic Saccharification of Rice Straw by Cellulase Produced by Trichoderma reesei under Solid State Fermentation". New York Science Journal 2010.
13. Frederick S. Archibald, A New Assay for Lignin-Type Peroxidases Employing the Dye Azure B, Applied And Environmental Microbiology, 1992, p. 3110-3116 Vol. 58, No. 9
14. Gupta, R. (2008). "Alkaline pretreatment of biomass of ethanol production and understanding the factors influencing the cellulose hydrolysis," Ph.D dissertation, Auburn University, Alabama.
15. Hu, Z., and Wen, Z. (2008), "Enhancing enzymztic digestaibility of switchgrass by microwave-assisted alkali pretreatment," Biochemical Engineering Journal 38(3),369-378.
16. Keshwani, D.R. (2009), "Microwave pretreatment for switchgrass for bioethanol production," PhD dissertation, North Carolina State University, Raleigh, North Carolina.
17. König, H., Fröhlich, J. and Hertel, H. (2005). Diversity and lignocellulolytic activities of cultured microorganisms. In: König, H. and Varma, A. (eds.). Intestinal Microorganisms of Termites and Other Invertebrates. Springer-Verlag, Berlin. pp. 272-302.
18. Leenakul W. and N. Tippayawong, Dilute Acid Pretreatment of Bamboo for Fermentable Sugar Production, Journal of Sustainable Energy and Environment 1 ,2010, 117-120
19. Maarten A, J Kootstra, Hendrik H Beeftink, Elinor L Scott and Johan PM Sanders, Optimization of the dilute maleic acid pretreatment of wheat straw, Biotechnology for Biofuels 2009, 2:31.
20. McCarthy AJ (1987). Lignocellulose-degrading actinomycetes. 1987. FEMS Microbiol. Lett. 46(2):145-163.
21. Mehdi Dashtban, Robert Buchkowski, Wensheng Qin. "Effect of different carbon sources on cellulase production by Hypocrea jecorina (Trichoderma reesei) strains". Int J Biochem Mol Biol 2011;2(3):274-286.
22. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428.
23. Narasimha G, sridevi A, Buddolla V, Subhosh CM, Rajsekhar RB (2006). Nutrient effect on production of cellulolytic enzymes by Aspergillus niger. Afr. J Biotechnol. 5(5): 472-476.
24. Naresh Sharma, K. L. Kalra, Harinder Singh Oberoi, Sunil Bansal, Optimization of fermentation parameters for production of ethanol from kinnow waste and banana peels by simultaneous saccharifi cation and fermentation, Indian J. Microbiol. (December 2007) 47:310–316.
25. Nutawan Yoswathana, Phattayawadee Phuriphipat, Pattranit Treyawutthiwat and Mohammad Naghi Eshtiaghi. "Bioethanol Production from Rice Straw" Energy Research Journal 1 (1): 26-31, 2010, ISSN 1949-0151.
26. Pezsa N and P.Ailer, Bioethanol production from paper sludge pretreated by subcritical water, Hungarian Journal of Industrail Chemistry , 2011, Vol.39(2)pp.321-324.
27. Pointing, S.B., Buswell, J.A., Vrijmoed, L.L.P. and Jones, E.B.G. (1999a) Extracellular cellulolytic enzyme profiles of five lignicolous mangrove fungi. Mycological research 1999a 103:(In press).
28. Rubeena M., Kannan Neethu, S. Sajith, S. Sreedevi, Prakasan Priji, K. N. Unni, M. K. Sarath Josh, V. N. Jisha, S. Pradeep, Sailas Benjamin, Lignocellulolytic activities of a novel strain of Trichoderma harzianum, Advances in Bioscience and Biotechnology, 2013, 4, 214-221.
29. Umbrin Ilyas, Abdul Majeed , Khalid Hussain, Khalid Nawaz,Shakil Ahmed and Muhammed Nadeem, Solid State fermentation of Vigna mungo for cellulase production by Aspergillus miger, World

Applied Sciences Journal , 2011,12(8): 1172-1178.
30. Vicuňa R (1988). Bacterial degradation of lignin. Enzyme Microb. Technol. 10:646-655.
31. Wood, T.M. and Bhat, M.K. (1988) Methods for measuring cellulase activities. In: Methods in Enzymology (W. Wood and S.J. Kellog, Eds.), Vol. 160, Academic press, New York, pp. 87-112.
32. Wyman C, Lynd L, Elander RT (1996) Likely features and costs of mature biomass ethanol technology. Appl Biochem Biotechnol 57–58:741–760.
33. Zimmermann W (1990). Degradation of lignin by bacteria. J. Biotechnol. 13(2-3):119-130.

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